to express the TfR1 did not contain the stalk, which had not been implicated in Tf binding prior to this study. Considering the close association of the N-terminal lobe with the stalk (Figures 1B and 1C) and its potential to affect Tf binding, future investigations may aim to examine its role in the Tf-TfR1 interaction. Finally, since the authors propose the importance of the N-lobe in modulating the binding of Tf to the TfR1, could the Fe-free conformation of this lobe be responsible for the lower affinity of apoTf for the receptor? Additional studies examining the crystal structure of apoTf-TfR1 complex will provide this answer.

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Catastrophic Kinesins: Piecing Together Their Mechanism by 3D Reconstruction

Kin Is, kinesins with an internal catalytic domain, depolymerize microtubules from both ends, and the KIF2C crystal structure presented by Ogawa et al. (2004, [this issue of *Cell*]) provides provocative evidence to support the theory that the highly conserved sequences are critical structural elements in these catastrophic kinesins.

Microtubules (MTs) are critical for many cellular processes such as cell division, cell movement, and vesicular trafficking. They are cytoskeletal polymers made up of α/β-tubulin heterodimers that assemble head to tail into protofilaments. Thirteen protofilaments associate laterally to form the hollowed structure of the MT. Essential for their cellular function, MTs have a unique property called dynamic instability that allows them to coexist in states of growth and shrinkage and to randomly interconvert between these two states (Mitchison and Kirschner, 1984). Unlike other kinesins, which use MTs as tracks, Kin I kinesins regulate dynamic instability by inducing catastrophes (Desai et al., 1999). A catastrophe occurs when a MT changes from growth to shrinkage by undergoing a conformational change at its ends. This conformational change consists of individual protofilaments transitioning from a straight to a curved conformation, resulting in tubulin dissociation and MT depolymerization. This curvature is due to the tubulin heterodimer taking on a relaxed or curved conformation. Recently, multiple studies of vertebrate MCAK and *Plasmodium* pKinI have attempted to unravel the molecular mechanism of Kin I-induced MT depolymerization (Hunter et al., 2003; Moores et al., 2002, 2003; Niederstasser et al., 2002; Ovechkina et al., 2002); however, this has been difficult due to the lack of 3D structural data. In this issue of *Cell*, Ogawa et al. present the long-awaited crystal structure of a Kin I, mouse KIF2C (Ogawa et al., 2004). Solving the KIF2C structure allowed them to identify several features that are distinct from motile kinesins. In addition, the authors have further advanced our understanding of the mechanism of Kin I MT depolymerization by docking the 3D structure of KIF2C to tubulin dimers in silico. This led to the identification of four important structural features that likely make crucial contacts with MTs. According to conventional kinesin nomenclature, these include: α4 (α helix 4), L2 (loop 2), L8 (loop 8), and the positively charged neck.

Strikingly, the authors find that KIF2C docked best to the 3D structure of a curved and not a straight protofilament (see Figure 5 in Ogawa et al. [2004]). This in silico docking places the KIF2C catalytic core central to the tubulin dimer at the intradimer groove with α4, a critical kinesin MT binding surface, nicely nestled into the interface. This places the bulk of KIF2C over α- and β-tubulin parallel to the MT axis with α4 laying perpendicular. This arrangement is consistent with the recent cryo-electron microscopy structures of pKinI on tubulin rings and structural predictions for *Xenopus* MCAK binding to MTs (Hertzer et al., 2003; Moores et al., 2002, 2003; Niederstasser et al., 2002). This helix, although conserved among kinesins, contains four completely conserved Kin I-specific amino acids and is longer than other kinesin α4 helices. Based on the modeling by the authors, these residues are predicted to stabilize a curved tubulin dimer by bridging the acidic C-terminal residues of β-tubulin (E hook) to the C terminus of α-tubulin (H11-H12 linker). This is consistent with previous results that the C-terminal residues of tubulin are essential for MCAK and pKinI depolymerization activity (Moores et al., 2002; Niederstasser et al., 2002).

The second structure predicted to be important in Kin I function is L2. L2 forms an extended rigid structure composed of anti-parallel β sheets centered on a loop that has the amino acid triplet, lysine (K), valine (V), and aspartic acid (D) at its apex. It is predicted to point down the MT axis and interact with α-tubulin. The authors have termed this structure the KVD-finger because within the docked structure of KIF2C on the protofilament it also makes contacts with the adjacent tubulin dimer at the interdimer groove. L8 is the third important structure and is positioned over and interacts with β-tubulin but on the other side of α4 in KIF2C. Interestingly, the KVD-finger and L8 can simultaneously contact the tubulin dimer but only in the curved conformation. The authors propose that these two structures stabilize a curved protofilament. These structures are therefore likely to be fundamental for Kin I destabilization activity and may act as protofilament tethers on either end of KIF2C. Interestingly, mutations of lysine, valine, and/or aspartic acid within the KVD-finger show that these residues are important, but not essential, for the depolymerization activity of KIF2C in vivo (Ogawa et al., 2004).

Ogawa et al. propose an additional and more exciting function for L8: the ATP hydrolysis sensor. KIF2C was crystallized with its nucleotide pocket in the open state in the presence of AMPPNP, an ATP analog. This is significant because the motile kinesin nucleotide pocket is thought to exist in the closed state in the presence of ATP. This closed conformation is proposed to induce the conformational changes needed for tight MT binding and to facilitate contacts for ATP hydrolysis, which moves kinesin along the MT (Kikkawa et al., 2001). Here, the authors propose that the Kin I nucleotide pocket only closes when L8 makes stable contacts with β-tubulin. This closed conformation of KIF2C would then be competent to hydrolyze ATP. Whether ATP is hydrolyzed on the MT end or on a free tubulin dimer remains unclear. In short, Ogawa et al. hypothesize that multiple Kin Is passively stabilize an already present curved conformation at MT ends through α4, L2, and L8. Alternatively, Kin Is may physically peel protofilaments because Kin Is curl and depolymerize stabilized MT substrates (Desai et al., 1999; Moores et al., 2002).

How this mechanism fits into the context of the MT, where lateral protofilament associations must be overcome, may depend on the orientation of the neck. The proximal part of the KIF2C neck was structured within the crystal, whereas the distal part of the neck was unstructured. The proximal part of the neck exits the catalytic core, forms a short helix, and then makes an approximate 90° turn followed by another helix. This turn points the neck perpendicular to the KIF2C and MT axis. This allowed Ogawa et al. to refine several suggested functions of the Kin I neck. First, the position of the neck could provide structural interference to prevent binding of the catalytic core to tubulin along the length of the MT (Hunter et al., 2003; Niederstasser et al., 2002; Ogawa et al., 2004). Second, the neck may provide a link to the MT to accelerate targeting to the ends (Hunter et al., 2003). Finally, Ogawa et al. propose that it functions at the end of a MT to prevent the reassociation of a curved protofilament with that of a straight protofilament. Specifically, they suggest that the distal part of the neck interacts with tubulin on the inside of the MT (M loop), which is only exposed at the ends. Support for these functions comes from studies of neckless versions of various Kin Is that depolymerize MTs and bind MT sidewalls less efficiently in vitro and in vivo (Hunter et al., 2003; Moores et al., 2002, 2003; Ogawa et al., 2004; Ovechkina et al., 2002). Confirmation of these structural interactions awaits the cocrystallization of a Kin I with MTs.

In summary, Ogawa et al. propose that the Kin I class-specific neck prevents tight MT sidewall binding while facilitating targeting to the MT end. Once at the end, the neck interacts with the lumen of the MT, preventing lateral associations between protofilaments. This allows the catalytic core to stably bind to the MT through α4. The interaction of the catalytic core with the MT through α4 allows the KVD-finger and L8 to stabilize a curved protofilament. Stabilization closes the nucleotide pocket making the Kin I competent to hydrolyze ATP. At this point, the MT is sufficiently destabilized to cause a catastrophe. Depolymerization ensues, and the Kin I either dissociates with a tubulin dimer or slips backward along the MT for the next round of MT destabilization. Overall, the authors have eloquently described the crystal structure of KIF2C and have proposed an exciting and testable model on how the class-specific α4, L2, L8, and neck function to bring about MT disassembly. With the crystal structure now in hand, the molecular mechanism of catastrophe-inducing kinesins will undoubtedly become clearer in the near future.

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